EXHIBIT A

...showed little regard to alterations in the ... increase in leaf thickness. ...dation changes the light micro- ...campestris. Consequently, it may ...distribution of PAR within the leaf may influence

...research areas

...Given that proteins are obvious candidates, more attention should be paid to particular chloroplast proteins and plasmalemma proteins since membrane damage will affect cell equilibrium. In addition, multiple stress effects need to be assessed to obviate the rather unnatural experimental situations where UV radiation is the only stress. More use of polychromatic light together with UV irradiation in laboratory experiments should be considered, since synergistic light effects may shift the sensitivity of the target sites. Field experiments provide the necessary high PAR levels, and for whole plant studies they are definitely an advantage over growth chamber

conditions. Greater care also needs to be taken with the UV fluences used so that results can be related to natural conditions.

1 M. M. Caldwell, R. Robberecht, R. S. Nowak and W. D. Billings, Differential photosynthetic inhibition by ultraviolet radiation in species from the arctic–alpine life zone, *Arct. Alp. Res.*, *14* (1982) 195–202.
2 J. F. Bornman and T. C. Vogelmann, Effect of UV-B radiation on leaf optical properties measured with fiber optics, *J. Exp. Bot.*, in the press.
3 G. Renger, M. Völker, H. J. Eckert, R. Fromme, S. Hohm-Veit and P. Gräber, On the mechanism of photosystem II deterioration by UV-B irradiation, *Photochem. Photobiol.*, *49* (1989) 97–105.
4 B. M. Greenberg, V. Gaba, O. Canaani, S. Malkin, A. K. Mattoo and M. Edelman, Separate photosensitizers mediate degradation of the 32-kDa photosystem II reaction center protein in the visible and UV spectral regions, *Proc. Natl. Acad. Sci. U.S.A.*, *86* (1989) 6617–6620.
5 Y.-P. Cen and J. F. Bornman, The response of bean plants to UV-B radiation under different irradiances of background visible light, *J. Exp. Bot.*, in the press.

J. of Photochemistry and Photobiology, B: Biology, 8 (1991) 340–341

Inflammation and photodynamic therapy

Isabel Freitas

Department of Animal Biology and CNR Centre for Histochemistry, University of Pavia, 27100 Pavia (Italy)

Tumour regrowth after photodynamic therapy (PDT) is usually explained in terms of the inadequate distribution of the sensitizer or of the photoactivating light and also of insufficient oxygenation. The acute inflammatory phase induced by PDT [1] is also likely to contribute to an improvement of the spared cells milieu and thus to tumour regrowth after PDT. Nevertheless, if duly faced, the inflammatory state pre-existing PDT or ensuing from it may perhaps be a double-edged sword susceptible to be turned to the therapist's advantage.

By studying an experimental tumour model, Ehrlich carcinoma, my group is currently investigating the inflammatory processes which occur as a response to the presence of necrotic areas and/or to the processes of neovascularization [2, 3]. We have observed the infiltration of a protein and neutral lipid-rich exudate into the extracellular space and, in particular, its accumulation against a "wall" of perinecrotic, vital, cells ("hypoxic cells") stuck against ghosts of necrotic cells. Under high magnification, cells in this region distant from blood vessels show an unsuspected burst of active proliferation; the density of mitoses is much higher than in the vicinity of the tumour capillaries, being only comparable with the density of mitoses at the tumour interface. This feature reminds us of how "beneficial" exudates usually are in normal inflammatory processes: the flow of inflammatory exudate brings oxygen and nutrients and thus helps to nourish the cells engaged in wound repair processes [4].

A

ly, changes in the internal spectral regime of leaves have been measured
its exposed to enhanced levels of UVB radiation [2].
uantification of the changes caused by UV radiation has been performed
a wide range of different methods, *e.g.* measurement of net and partial
synthesis, flash-induced absorption changes, fibre optic microprobes
measurement of the internal photosynthetically active radiation (PAR)
ves, phytoluminography and monitoring of chemiluminescence, to
on a few.

cently proposed target sites of UVB radiation

review of our knowledge so far on the possible targets and mechanisms
radiation damage in higher plants shows that certain trends are emerging
iteresting developments have occurred as a result of the use of a wide
of methods. Work on the different partial reactions of the photosynthetic
on transport chain has given some varying results with regard to sensitive
rgets. Several different target sites have been proposed. These include
action centre of photosystem II, (PS II), the light-harvesting complex,
ceptor side of PS II and the donor side of PS II. Detailed studies of
have shown that the functional integrity between the water-splitting
lex and P680 on the oxidizing side of PS II is impaired with exposure
B radiation [3]. However, the reducing side of PS II is also a sensitive
rget, as shown by Greenberg *et al.* [4]. They found that the chloroplast
in, D_1 (also designated Q_B or 32 kdalton protein), which is the primary
ng site of atrazine herbicides, is rapidly degraded on exposure to UV
ion. Exposure to sunlight without a UV-blocking filter increased the
dation rate by 30%. This degradation of Q_B is thought to be mediated
e semiquinone anion radical.

portance of visible light

Numerous experiments have shown that plants may respond differently
grown in greenhouses or growth chambers with suboptimal PAR as
sed to those exposed to natural sunlight. The interactive role of different
s of PAR with UVB radiation was especially evident in a recent study
Phaseolus vulgaris [5]. These bean plants were grown under three
s of visible light (230, 500 and 700 μmol m^{-2} s^{-1}, referred to as LL,
r HL) with or without enhanced UVB radiation. Plants grown under HL
itions were most resistant to UVB radiation, whereas LL conditions
nced response to UV radiation. ML grown plants in general showed a
onse intermediate between the other two light treatments. Under all three
itions leaves responded with a significant increase in leaf thickness, a
onse which, together with pigment changes, appears to influence internal
gradients and possibly photosynthesis. However, increase in leaf thickness

least in LL grown plants, a result of suboptimal growth conditions.
larly, induction of UVB-absorbing compounds was not pronounced under
UVB radiation. In LL grown plants leaf reflectivity, measured with
cated optical fibres, was highest in the blue and red regions of the
trum, a phenomenon likely to decrease the penetration of PAR.

hanges in internal PAR after UV radiation

In order to measure the internal spectral regime of leaves and other
t organs, the sample is irradiated with a light source and a small quartz
(1–5 μm diameter) is advanced into the leaf using an automatic stepper
or. The other end of the fibre is attached to a holder fitted to a
troradiometer for measuring the light striking the fibre as the tip is
nced through the leaf. This method is used to define more precisely
re in the leaf changes take place after exposure to different environmental
itions.
In a recent study, two species of *Brassica*, one from a northern latitude
in (*B. campestris*) and the other from southern latitudes (*B. carinata*),
grown under either high visible light (1800 μmol m^{-2} s^{-1}) or additional
olemental UVB radiation [2]. *B. campestris* was most sensitive to UVB
ation, responding with a 45% increase in leaf thickness as well as with
eases in chlorophyll (Chl) fluorescence and Chl content. Together with
e changes, the internal PAR of the leaves changed with respect to the
attenuated. However, it was

From a single dose PDT viewpoint, the negative side of the inflammation which it causes may thus be tumour relapse if destruction of neoplastic cells has not been totally achieved. Several positive aspects may, however, be found for pre-existing inflammation [5], namely the exudate may help to deliver protein-bound photosensitizers to the inner areas of the tumour, its dissolved oxygen may increase the local pO_2 levels of areas distant from the vasculature with respect to the pO_2 values calculated simply in terms of oxygen diffusion and consumption, and tumour fibrin loci (obtained by clotting of extravasated fibrinogen) may provide further binding sites for the photosensitizers. If, as will probably occur, the first PDT dose is insufficient for complete eradication of the tumour, we should not discard the idea of planning the second dose in such a way as to exploit the ensuing inflammatory phase, in particular the possibility of obtaining widespread tissue distribution of the drug transported by the exudate proteins, higher cellular uptake of the drug due to the presence of a highly proliferative population, and improved oxygenation brought about by the plasma-like fluid. The negative aspects are the haemorrhage, due to the collapse of blood vessels, which will hinder light penetration in the tissue, and the possibility that two close PDT doses might not be well tolerated by the patient.

In my opinion the inflammatory state must be faced, whatever the result of the balance between its positive and negative sides might be, and despite the addition of a further complexity to an already complex modality of cancer treatment.

1 C. Zhou, Mechanisms of tumor necrosis induced by photodynamic therapy, *J. Photochem. Photobiol. B: Biol.*, *3* (1989) 299–318.
2 I. Freitas, S. Barni, C. Del Rio, V. Bertone, M. Parente, G. F. Baronzio, P. Pontiggia and G. Roveta, Neovascularization patterns in Ehrlich carcinoma. An electron microscopy study, *Med. Biol. Environ.*, *18* (1990) 375–381.
3 I. Freitas, Lipid accumulation. The common feature to photosensitizer-retaining normal and malignant tissues. *J. Photochem. Photobiol. B: Biol.*, *7* (1990) 359–361.
4 J. R. Anderson (ed.), *Muir's Textbook of Pathology*, Edward Arnold, London, 1987, pp. 4.1–4.38.
5 H. V. Dvorak, Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing, *New Engl. J. Med.*, *315* (1986) 1650–1559.

J. of Photochemistry and Photobiology, B: Biology, 8 (1991) 341–342

Comment on "A guide to the terminology of hematoporphyrin-catalyzed photosensitization" (Michael A. J. Rodgers, *J. Photochem. Photobiol., B: Biol.*, *5* (1990) 525) — a comment

David Kessel
Wayne State University School of Medicine, Detroit, MI 48201 (U.S.A.)

Rodgers suggested that supplies of hematoporphyrin derivative (HPD), Photofrin II, etc. be discarded since no rational scientist would carry out studies on such an undefined mixture. I argued for a better appreciation of the nature of this mixture, but the complexity of HPD may yet save us from

the Food and Drug Administration which must approve all drugs for human use in the United States of America.

While development of new photosensitizers has continued at a rapid rate, none of these agents has yet reached clinical trials. With a safe drug already at hand, it is difficult to formulate a rationale for human studies on potentially toxic new dyes. Since we know that the components of HPD are safe, drug development based on synthesis of ether and ester dimers of hematoporphyrin and its dehydration products may therefore both expedite the approval process and save us from the need to discard all of the HPD, Photofrin, Photofrin II, Y-HPD, Photosan and assorted impurities which have been accumulating during the past 30 years.